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A Tale of Too Many Centrosomes

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Having the correct number of centrosomes is crucial for proper chromosome segregation during cell division and for the prevention of aneuploidy, a hallmark of many cancer cells. Several recent studies (Basto et al., 2008; Kwon et al., 2008; Yang et al., 2008) reveal the importance of mechanisms that protect against the consequences of harboring too many centrosomes.

When cells divide, it is essential that they segregate their newly duplicated chromosomes into two equal sets. Missegregation not only distorts the number of chromosomes in the daughter cells but also elevates or diminishes the expression of genes critical for cell viability and growth. The equal segregation of sister chromatids at mitosis is directly linked to the organization of the microtubule-based mitotic spindle that guides chromosome separation. The microtubule organizing center of the cell, or centrosome, duplicates during interphase of the cell cycle, when the cell is between rounds of division. At the start of mitosis, the duplicated centrosome pair separates. Microtubules emanating from each of the centrosomes associate together to form a bilaterally symmetrical mitotic spindle with each spindle pole organized around a single centrosome. Duplicated sister chromatids aligned at the midpoint between the two spindle poles become attached to the microtubules emanating from each pole; at anaphase, the sister chromatids separate and migrate to opposite spindle poles. Having only two centrosomes per cell in mitosis ensures equal partitioning of the two sets of sister chromatids, thus maintaining genomic continuity between the mother and daughter cells. Despite the potential for chromosomal loss when a cell has more than two centrosomes, centrosome amplification can occur during normal development. Normal cells with more than two centrosomes arise, for example, during postnatal differentiation of liver hepatocytes (Margall-Ducos et al., 2007), fusion of trophoblastic cells in the placenta (Huppertz et al., 2006), or fusion of myoblasts during normal differentiation (Pajcini et al., 2008). Abnormal cells such as cancer cells also frequently show early amplification of centrosome number. Indeed, centrosome amplification is linked to increased chromosomal instability and tumor progression (reviewed in Srsen and Merdes, 2006), although whether chromosome instability drives tumorigenesis or is merely a consequence of cellular transformation remains debatable. Nonetheless, the existence of viable cells harboring more than two centrosomes suggests the presence of mechanisms that allow cells to deal with the challenge of maintaining bipolar symmetry during mitosis despite an increased number of centrosomes. Centrosome amplification increases the number of spindle poles to which sister chromatids can become attached, thus elevating the potential for unequal chromosome segregation (Saunders et al., 2000). It has been proposed that the survival of cancer cells with multiple spindle poles is influenced by their ability to undergo bipolar division regardless of the extra spindle poles (Brinkley, 2001). Three recent studies by Basto et al. (2008) in *Cell*, Yang et al. (2008) in *Nature Cell Biology*, and Kwon et al. (2008) in *Genes and Development* provide fresh insights into the mechanisms that prevent the deleterious consequences of centrosome amplification and present evidence for a direct link between increased centrosome number and cancer.

One mechanism for minimizing the damaging effects of extra centrosomes, which has been observed in some cells, is the clustering of the centrosomes into two centrosomal groups during mitosis to allow the formation of a bipolar spindle (Brinkley, 2001; Quintyne et al., 2005). In their new work, Yang et al. (2008) studied cultured human retinal pigmented epithelial cells (RPE1) treated with a reversible inhibitor of cell division to generate daughter cells that each possess two sets of centrosomes and chromosomes (Yang et al., 2008). Despite the double burden of chromosomes and centrosomes, these daughter cells could still divide. Remarkably, whereas all of these daughter cells initially formed multipolar spindles, as might be predicted, ~74% of the cells showed clustering of the centrosomes into groups to form bipolar spindles during metaphase. Similar centrosome clustering was observed by Basto et al. (2008), who studied the effects of amplifying centrosome number by overexpressing the centriolar replication factor SAK/PLK4 (*SAKOE*), which drives centrosome overduplication in the fly *Drosophila melanogaster*. Analysis of fly larval brain tissue from mutant *SAKOE* flies showed that ~93% of fly cells with extra centrosomes formed bipolar spindles during metaphase. Thus, normal metazoan cells can respond to the problems presented by extra centrosomes by clustering centrosomes at metaphase to produce a bipolar spindle. Basto and colleagues also identified another event that minimizes the negative impact of extra centrosomes. They observed that some of the extra spindle poles in the *SAKOE* fly cells failed to organize microtubules, thereby allowing the spindle to be operationally bipolar even with multiple centrosomes present. The study by Kwon et al. (2008) reveals additional actin-associated mechanisms for centrosome clustering. Using a genome-wide screen of mutations in cultured *Drosophila* S2 cells, Kwon et al. identified a diverse array of proteins involved in clustering, including both microtubule- and actin-associated proteins. When cells were treated with compounds that limit

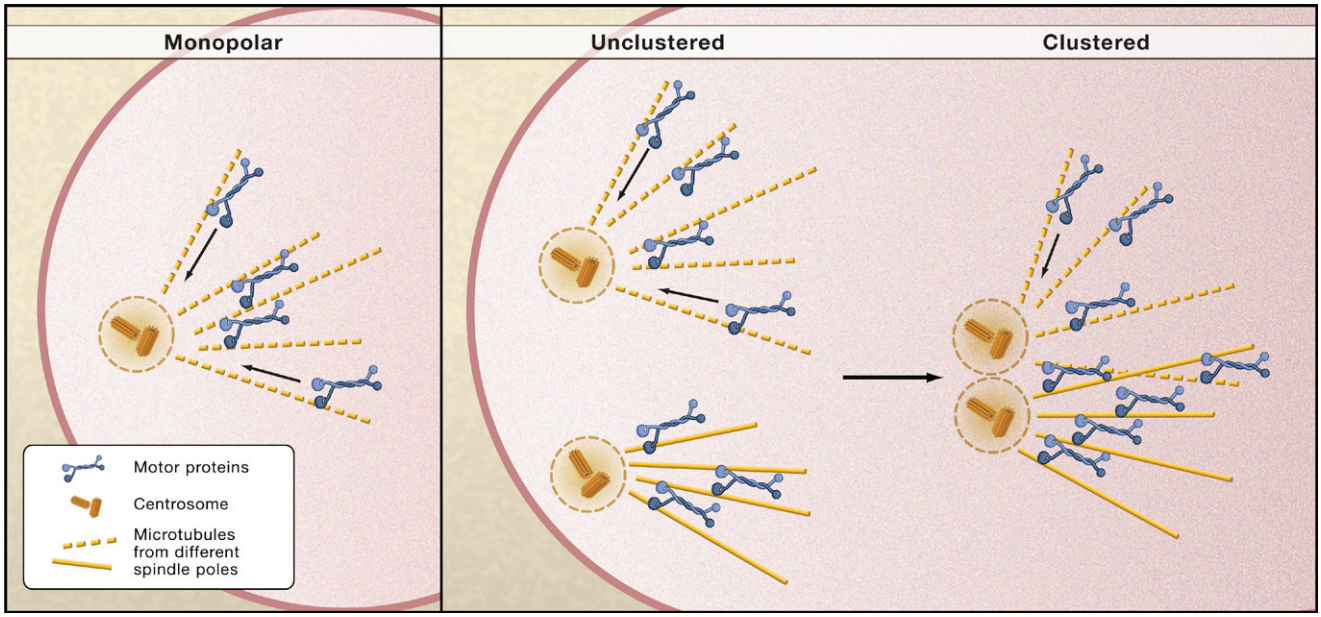

Figure 1. A Cellular Response to Extra Centrosomes

One possible mechanism for minimizing the impact of additional centrosomes in the cell involves the clustering of centrosomes by molecular motors. Molecular motors are required to focus the microtubule fibers at the centrosomes in normal cells (Hatsumi and Endow, 1992; Merdes et al., 2000). Focusing may occur by movement of the motor toward the centrosome (arrow), a process that crosslinks the microtubules near their ends. The motors could crosslink microtubules directly (shown) or could bind to scaffolding intermediates such as the spindle protein NuMA (not shown) to increase crosslinking. In cells with extra centrosomes, the centrosomes could be clustered together to prevent formation of extra mitotic spindles by crosslinking of microtubules emanating from different poles.

actin polymerization, centrosomal clustering was diminished in both fly and mammalian cells. Kwon et al. were further able to manipulate spindle polarity by coating glass coverslips with different geometric patterns of the extracellular adhesion protein fibronectin, suggesting that cells are able to use both spindle microtubule and cortical actin molecular forces to control centrosome distribution in the spindle.

It is now confirmed that intracellular molecular motors can induce the coalescence of excess centrosomes to allow the formation of a normal bipolar spindle. Earlier work demonstrated that this clustering of extra centrosomes was dependent on cytoplasmic dynein, a microtubule-specific motor protein. When dynein was inhibited in diploid fibroblasts, clustered centrosomes dissociated from one another and became unclustered (Quintyne et al., 2005). Consistent with the involvement of motor proteins in this process, Basto et al. and Kwon et al. now report that in *Drosophila* the kinesin 14 motor protein Ncd is essential for centrosome clustering. In *SAKOE,ncd* double mutant flies, the number of multipolar spindles increased, cell division was no longer symmetrical, and fly development was slowed. Ncd is known to be important for maintaining centrosome organization (Hatsumi and Endow, 1992). Similarly, cytoplasmic dynein is also needed to maintain normal centrosomal structures in mammalian cells (Merdes et al., 2000). Therefore, it appears that the cell uses the same molecular mechanisms for clustering centrosomes as it does for maintaining the structure of a single centrosome. Because both the Ncd and dynein motors move toward the minus ends of microtubules, it is possible that spindle motors may cluster centrosomes together by crosslinking microtubules anchored at separate centrosomes (Figure 1). The motors may crosslink microtubules directly or indirectly through an intermediary such as the essential mammalian spindle protein NuMA (Quintyne et al., 2005; Wong et al., 2006).

Another mechanism that may contribute toward resolving the problems posed by excess centrosomes is the spindle assembly checkpoint (SAC). This mitotic sensor monitors sister chromatid attachment to the spindle microtubules to ensure that each chromatid of the pair attaches to microtubules belonging to opposite poles. During normal cell division, SAC blocks anaphase before all of the chromatids are bound by inhibiting the E3-like ubiquitin ligase anaphase-promoting complex (APC). The question of whether SAC is also activated by spindle structural abnormalities has been controversial. Monopolar spindles cause a marked delay at the onset of mitosis, which could be explained by the failure of chromosomes to attach to separate poles (Jensen et al., 1987). One study found that sea urchin zygotes and mammalian cultured PTK1 cells harboring multipolar spindles did not show delayed entry into anaphase (Sluder et al., 1997).

Yang et al. (2008) now report that RPE1 cells with multipolar spindles take twice as long as control cells to enter anaphase. Similarly, Basto and colleagues showed that the mitotic index of multicentrosomal *SAKOE* mutant cells in fly brain tissue increased modestly (Basto et al., 2006). This suggests that SAC may indeed be linked to mechanisms of solving the centrosome amplification problem. To test whether SAC is required for the observed mitotic delay, Yang et al. injected a dominant-negative form of the SAC positive regulator Mad2 into the RPE1 cells containing multipolar spindles and found that it eliminated

the mitotic delay. Basto et al. saw similar effects when they depleted Mad2 in *SAKOE* fly larvae: the mitotic index returned to a near normal frequency in the absence of Mad2 (Basto et al., 2008). Kwon et al. also made similar observations in cultured *Drosophila* S2 cells. In addition, Basto et al. report that *SAKOE*,*mad2* double mutant fly larvae developed faster than wild-type flies but died in the pupal stage, suggesting that SAC is required for the protective delay in anaphase that occurs in cells with centrosome amplification. An active SAC appears to compensate for a deficiency in centrosome clustering as *SAKOE*,*ncd* mutant cells (which cannot cluster their extra centrosomes) showed a marked increase in mitotic index but were still able to divide with apparently bipolar spindles and had only a low level of aneuploidy (Basto et al., 2006). These findings are consistent with a SAC-dependent delay that allows other mechanisms to cluster the centrosomes, enabling a bipolar spindle to form in the absence of Ncd. These data do not exclude the possibility that Mad2 has additional functions unrelated to SAC that are required for development in the presence of additional centrosomes.

Some mechanisms of centrosome amplification, such as a block in cytokinesis, have the added effect of also amplifying the number of chromosomes in the cell. Under those circumstances, it can be difficult to distinguish whether a delay in anaphase is due to an aberrant increase in the numbers of centrosomes or chromosomes. Thus, creative approaches are needed to increase the number of active centrosomes without increasing ploidy to distinguish the effects of these two amplification events.

The *Drosophila* protein DSas-4 is required for replication of the centriolar core of the centrosome (Basto et al., 2006). *Dsas-4* fly mutants cannot form centrosomes but can survive until birth. Interestingly, *SAKOE*,*Dsas-4* double mutant flies showed only a small developmental delay similar to that in flies harboring the *Dsas-4* mutation alone. This suggests that centrosomes are needed to induce the developmental delay in the *SAKOE* cells. Meanwhile, in their study, Yang et al. microirradiated one centrosome in RPE1 cells treated with a cytokinesis inhibitor. In these ablated cells now harboring only a single centrosome, the mitotic delay was reduced by half. Furthermore, Yang and colleagues found that the fusion of diploid RPE1 cells to enucleated cytoplasts (cells lacking nuclei), a process that adds a single centrosome but no chromosomes, caused the same mitotic delay as blocking cytokinesis. Together, these results prove that the mitotic delay is a consequence of increased centrosome number. However, we do not yet know if the mitotic delays observed under all of these circumstances are still SAC dependent.

Although additional centrosomes were required for the mitotic delay in cells with elevated centrosome numbers, it is still uncertain whether SAC specifically recognizes the presence of another centrosome or if extra centrosomes simply delay chromosome attachment enough to activate SAC through the established chromatin attachment surveillance pathway. Intriguingly, Mad2 localizes to centrosomes (Howell et al., 2000), but there is no evidence at this time that extra centrosomes can delay anaphase once all chromatids have bound to opposing spindle microtubules. Indeed, an anaphase delay was not observed in PTK1 cells with multipolar spindles once all chromatids were attached (Sluder et al., 1997). However, regardless of whether extra centrosomes activate SAC directly or not, the SAC-initiated delay is required for cells to cluster their centrosomes. For example, multipolarity and aneuploidy both increased dramatically in *SAKOE*,*mad2* fly brain cells (Basto et al., 2006).

So are these mechanisms of creating bipolar spindles in the presence of extra centrosomes sufficient to prevent chromosome missegregation? Basto et al. tested this presumption by examining the frequency of aneuploidy and aborted development in the *SAKOE* flies harboring excess centrosomes. Although the frequency of aneuploidy doubled in the mutant flies, it still remained at ~1.75%, a remarkably low frequency for cells harboring twice the normal number of this key mitotic organizer. Surprisingly, flies with amplified numbers of centrosomes still developed normally, albeit with a substantial delay and with ~60% embryonic lethality. These flies showed centrosome amplification and a stable diploid genome over many generations. Thus, at least in *Drosophila*, normal cells can correct for extra centrosomes and the presence of multipolar spindles efficiently enough to secure their genome as needed for development.

Centrosomal amplification is unusual in most nontransformed cell types. So why has a mechanism evolved to allow cells to cope with such an infrequent event? One possibility is that centrosomal amplification is especially deleterious during meiosis or early development when asymmetric cell division is common. Why is the maintenance of asymmetric division so important? Stem cells divide asymmetrically to generate one self-renewing stem cell and one differentiating cell. Disruption of asymmetric cell division in fly mutants is known to produce tumors when larval brain tissue from these mutant flies is implanted into the abdomen of adult flies. The implanted tissue produced tumors that were aneuploid and abnormal in centrosome number and morphology, killing the adult flies within 2 weeks (Caussinus and Gonzalez, 2005). Consistent with the effect of extra centrosomes on asymmetric cell division, *SAKOE* mutant flies seemed especially sensitive to the extra centrosomes during early stages of development and showed asymmetric division defects during neuronal stem cell differentiation (Basto et al., 2006). Centrosomes are known to play an important role in achieving asymmetry through proper alignment of the spindles along the apicobasal axis (reviewed in Yamashita and Fuller, 2008). In this context, mother and daughter centrosomes are also asymmetric in terms of their microtubule nucleation capacity, size, molecular composition, and ability to localize fate-determining mRNAs (Yamashita and Fuller, 2008). (A “centrosome sorting” mechanism has been proposed in mollusks for the distribution of mRNAs involved in developmental patterning, in particular genes encoding determinants of the anterior-posterior axis, or encoding secreted molecules and proteases involved in Dpp signaling.) As for nucleation capacity, the mother centrosome appears to be dominant, nucleating more microtubules than the daughter centrosome. The daughter centrosome only gains this microtubule-nucleating ability when it migrates to the opposite pole of the cell at the onset of mitosis. Previous studies found that in

SAKOE flies, there was no recognizable dominant centrosome, indicating that centrosomal asymmetry is likely also disrupted in the *SAKOE* fly neuroblasts with extra centrosomes (Basto et al., 2006). Indeed, only 60% of the *SAKOE* cells achieved the proper spindle alignment as compared to 95% of wild-type cells, with 15% of spindles in *SAKOE* cells showing an alignment never seen in wild-type flies. Hence, the presence of extra centrosomes appears to interfere with the spindle orientation and asymmetric division important for early development. To further determine the consequences of centrosome amplification on asymmetric cell division, Basto et al. transplanted fly larval brain tissue from *SAKOE* mutant flies into wild-type adult hosts. Transplanted wild-type brain tissue did not overproliferate or form tumors, but 14%–20% of the *SAKOE* tissue transplants formed tumors in wild-type hosts. Importantly, tumorigenesis occurred without extensive aneuploidy in the transplanted cells, although whether the tumor cells in the host were aneuploid was not reported.

These findings are important because they demonstrate that centrosome amplification, in the absence of other known changes, is sufficient to promote tumor formation. Intriguingly, Basto et al. also observed that 9% of the larval neuroblasts with extra centrosomes divided symmetrically instead of asymmetrically, leading to a slight but significant elevation of the stem cell pool in the larval brain. This phenomenon was never observed in wild-type neuroblasts and could potentially account for the tumorigenesis seen in flies receiving *SAKOE* larval brain transplants. It will be interesting to determine in future studies if the presence of extra centrosomes in cells that normally divide symmetrically can also induce tumors. Future analysis will be required to determine whether centrosome clustering can minimize the tumor potential of the *SAKOE* transplants. For example, would mutations in *Mad2* or *Ncd* enhance the rate of tumor formation after *SAKOE* transplantation? Do tumors resulting from the *SAKOE* transplants lose the ability to cluster centrosomes? Kwon et al. showed that suppressing centrosome clustering by depletion of HSET/Ncd inhibited growth of cultured human cancer cells with amplified centrosomes but not cancer cells lacking centrosome amplification or noncancerous NIH 3T3 cells. These findings add to earlier results suggesting a link between cytokinesis defects and tumorigenesis in p53-deficient mouse mammary epithelial cells (Fujiwara et al., 2005). It will now be important to determine whether centrosome amplification is sufficient to promote tumorigenesis in this mouse model or whether polyploidy is also required. More generally, the question is whether the common link between polyploidy and cancer (reviewed in Ganem et al., 2007) is actually an indication of a link between centrosome amplification and cancer, given that polyploidy frequently accompanies centrosome duplication. The exciting three new studies provide fresh insights into the molecular mechanisms that are activated in response to centrosome amplification. Future work will be required to test the relevance of these exciting findings to mouse models of tumorigenesis and the cellular changes found in clinical tumor samples from human cancer patients.

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